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Amino acids content in 'Tempranillo' must from three soil types over four vintages

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Summary

Amino acids are the main grape nitrogen compounds and the principal source of N for yeasts, being precursors of several volatile compounds. Therefore, N compound concentrations in musts can affect sensorial characteristics of wines. The aim of this study was to analyse the influence of N-NO_3^- and N-NH_4^+ contents from different soils on profile and content of amino acids in 'Tempranillo' grapes. In order to determine this soil influence on must quality, three plots were selected in AOC Rioja, classified as *Fluventic Haploxerepts*, *Typic Calcixerepts*, and *Petrocalcic Palexerolls*. The results showed that amino acids and yeast assimilable nitrogen (YAN) content allowed us to differentiate samples from the three soils, and in each soil type, samples of each season. In general, must contents of total amino acids and some of them, as alanine, threonine, and tyrosine, were more influenced by soil type; YAN, proline, histidine, serine, and glycine concentrations mainly depended on the interaction soil-vintage. In conclusion, free amino acids concentration could be a tool to differentiate musts coming from different soils.

Key words: soil N-NO_3^- availability; N-NH_4^+ availability; grape; terroir; nitrogen; *Vitis vinifera*.

Introduction

In Mediterranean conditions, traditionally, grapevines have been planted in marginal or even poor soils, probably because they require less water and nutrients to produce quality berries than many other fruit crops. Climate, soil and vine (rootstock and cultivar) are the main factors that have been considered determinants to define the grape and wine characteristics and quality (Seguin 1986, Van Leeuwen *et al.* 2004). Nowadays, the effects of these factors on growth and development of the vine vegetative and reproductive organs and subsequent effects on grape and wine can be modified or improved using different viticultural and oenological techniques. However, it is well known that water and main nutrients (especially nitrogen (N)) status during critical moments of vine phenological cycle influence vine yield, berry composition and grape sanitary status, affecting the correct development of fermentation processes and, ultimately, wine quality (Garde-Cerdán *et al.* 2009, Martínez-Gil *et al.* 2012).

Since grapevines cannot use atmospheric N gas (N_2) directly, their roots have to uptake N ions dissolved in the soil solution. This grapevine N uptake depends largely on soil properties, especially on both soil organic matter content and its mineralization speed. The latter depends on soil humidity, temperature, pH, aeration and the C/N ratio of organic matter (Van Leeuwen *et al.* 2000). In most aerobic soils, nitrate (NO_3^-) is the primary source of N for grapevines, due to the rapid nitrification of NH_4^+ derived from organic sources (Keller *et al.* 2001). When grapevine N status is high, grape composition is primarily influenced by the consequences of increasing grapevine growth (e.g. sink-source relationships, canopy microclimate). Therefore, high N status may disrupt vine balance, leading to a limited supply of carbohydrates if the vine becomes overcropped or excessively vigorous (Bell and Henschke 2005). Kliewer (1977) concluded that high N status resulted in reduced grape colour and total soluble solid concentration because photosynthates had been diverted away from carbohydrate metabolism to amino acids and protein synthesis and storage. Bell (1994) indicated that the conversion of NO_3^- to other N compounds is limited at high N levels.

The amino acid concentration in must has been reported as a good parameter to evaluate grapevine N status (Linsenmeier *et al.* 2008). *Saccharomyces cerevisiae,* the main yeast that carried out the alcoholic fermentation, preferentially uses simple N sources such as ammonium and free amino acids (Monteiro and Bisson 1991, Jiranek *et al.* 1995). However, the secondary amino acids, such as proline, are not metabolised to a large extent under usual winemaking conditions (Ingledew *et al.* 1987). Therefore, N compounds have a significant impact on fermentation development, as N-deficient musts can cause slow or stuck fermentations. Moreover, amino acids are precursors of volatile fermentative compounds, especially higher alcohols and esters (Hernández-Orte *et al.* 2005, Garde-Cerdán and Ancín-Azpilicueta 2008). These compounds are among the most important ones that constitute the "fermentation bouquet" of wines (Romano *et al.* 2003), therefore, N compounds concentration in the must can affect wine quality.

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The main objective of this study was to assess the influence of the soil N content on the N composition of 'Tempranillo' grapes. For this, assuming homogeneous climatic conditions in the area, the N levels in the soil alleyways of the three experimental plots were measured at different soil depths, and quality parameters, yeast assimilable N (YAN) and the concentration of 22 amino acids in the must were determined.

Material and Methods

Vineyards site description and experimental design: The experiment was conducted during four consecutive seasons (2013-2016) in a vine growing area formed by a river and torrential platforms of the Najerilla valley (AOC Rioja), northern Spain. The three plots have similar lithology, and loam or sandy loam texture (Tab. S1). Each experimental plot represents a soil type that was classified, according to American soil taxonomy (Soil Survey Staff 2010), as *Fluventic Haploxerepts (FH), Typic Calcixerepts (TC)*, and *Petrocalcic Palexerolls (PP).* According to the UNESCO aridity index (1979), the climate in the area is semiarid Mediterranean. Weather conditions of the four seasons were recorded by a meteorological station of the Regional Agroclimatic Service (www.larioja.org/siar) situated on the vineyard area (lat., 42°27'42.75'N: long., 2°42'45.66'W, and altitude 465 m a.s.l.). In Tab. 1, data of annual precipitation, average annual temperature, potential evapotranspiration (FAO-Penman Monteith) and grapevine growing cycle (April-October) precipitation and evapotranspiration, during 2013, 2014, 2015 and 2016 seasons, are shown.

In each plot, three adjacent rows with 50 grapevines each (*Vitis vinifera* L. 'Tempranillo' grapevines grafted on 110 Richter rootstock) were marked as repetitions. Planting density was 2,900-3,000 plants·ha^{-1}. All of them were spur pruned to twelve buds per vine and in spring, all water shoots were pulled off.

Soil cover management between rows was the same for the three experimental vineyards: conventional tillage, disked every 4 to 6 weeks at 0 to 15 cm depth, during the growing season (February-August), and the strip under the vines was kept free of weeds by means of herbicide. Grapevine canopy management and control of grapevine pests and diseases by chemical spraying were carried out by machines and the crop was manually harvested. In certain years (2013 and 2015), the wine grower of FH plot, following the guidelines of the AOC Rioja, did cluster thinning in dates close to the harvest. Only in 2014 season, mineral soil fertilizer NPK 8:0:18 (200 kg·ha^{-1}) was applied to all plots, but during the experiment no more N-fertilizer was used either to the soil nor *via* foliage spray. Water was not supplied by irrigation and the vine water status depended on collected rainfall.

Soil sampling and soil N-NO_3^- and N-NH_4^+ analysis: Composite soil samples were randomly collected by means of an Edelman® auger, at bloom (June), in the alleyways of each plot at 0-15, 15-30, and 30-45 cm depth. The soil samples were air dried and then ground and sieved to 2 mm. The percentage of coarse fragments (> 2 mm) was determined in each sample. Soil N-NO_3^- and N-NH_4^+ were determined by colorimetry (Pérez-Álvarez *et al.* 2015a). In order to express N-NO_3^- and N-NH_4^+ content in kg N·ha^{-1}, soil bulk density determined by the core method was employed (Grossman and Reinsch 2002), as well as the soil percentage of coarse fragments, at the three soil depths studied (0-15, 15-30, and 30-45 cm).

Yield components, berry sampling and must oenological parameters analysis: At harvest (October), in each plot, random samples of approximately 600 grape berries were collected from 20-25 grapevines distributed randomly throughout each repetition (row) at their optimum technological maturity (*i.e.* when the weight of 100 berries remained constant and the probable alcohol reached 12-13 %). The number of clusters per vine and grape yield produced per row were recorded in order to calculate the average cluster weight (kg grape·vine^{-1}). In the laboratory, 100 berries of each repetition were counted and weighted. Later, all the berries of each repetition were crushed together to obtain the must. One part of the must was frozen (- 20 °C) and kept until the YAN and the free amino acids determination; the other part of the must was employed to determine the oenological parameters.

Musts were physico-chemically characterized by determining pH, probable alcohol, total acidity, malic acid, colour intensity and potassium according to the OIV methods (2014). Tartaric acid was analyzed by Rebelein method (Lipka and Tanner 1974). Total polyphenol index (TPI) was determined by measuring the absorbance at 280 nm after conventional dilution of samples (Somers and Evans 1974). Total anthocyanins were analyzed by decolouring using sulphur dioxide (Ribéreau-Gayon and Stonestreet 1965).

Determination of free amino acids and YAN: Analysis of amino acids in must was performed according to the method described by Garde-Cerdán *et al.*

Table 1

Climatic conditions during the four seasons (2013-2016) in the vineyard area

	2013	2014	2015	2016
Average annual temperature (°C)	12.0	13.1	12.4	13.2
Annual precipitation (mm)	678	559	465	481
April–October precipitation (mm)	314	235	171	118
Potential evapotranspiration (mm)	880	958	956	964
April-October potential evapotranspiration (mm)	710	771	757	802

(2014). Free amino acids were analyzed by reverse phase HPLC-FLD/DAD (Agilent 1100 Series, Palo Alto, USA). The identification of N compounds was carried out by comparing their retention times and their UV-Vis spectra with those of pure reference standards (Sigma-Aldrich, Madrid, Spain). Quantification of amino acids was performed with an internal standard method and was done using the calibration graphs of the respective standards ($R^2 > 0.94$) in 0.1 N HCl, which underwent the same process of derivatization as the samples. The amino acids identified and quantified were proline (Pro), arginine (Arg), γ-aminobutyric acid (GABA), glutamine (Gln), glutamic acid (Glu), alanine (Ala), histidine (His), tryptophan (Trp), threonine (Thr), serine (Ser), aspartic acid (Asp), cysteine (Cys), phenylalanine (Phe), valine (Val), leucine (Leu), tyrosine (Tyr), asparagine (Asn), citrulline (Cit), glycine (Gly), isoleucine (Ile), methionine (Met), and lysine (Lys). Unit for free amino acids was milligrams of N per kilogram of whole berries (as mg $N \cdot kg^{-1}$ for conciseness). Total free amino acids content was determined by summing the individual free amino acids values. Yeast assimilable N values (YAN) in must (mg $N \cdot kg^{-1}$) were determined according to the method described by Aerny (1996).

Statistical analysis: Data were analyzed using SPSS Version 21.0 statistical package for Windows (Chicago, USA). The variance analysis model (ANOVA) was used for individual sample comparisons of the oenological parameters, yield components and N compounds at $p \leq 0.05$, with separation of means by the Duncan test. Also, percentage of variance attributable to the soil type, season and their interaction was made. Discriminate functions were performed with amino acids concentration using the statistical software referenced.

Results and Discussion

Soil characteristics: In accordance with the low organic-matter content of the samples, approximately 1 % (Tab. S1), the soil N-levels were low (Tab. 2), as occurs in soils of the same vineyard area (Pérez-Álvarez *et al.* 2013, 2015a, Peregrina *et al.* 2010). Effective depth, water reserve available values, texture, and calcium carbonate content in the subsoil were quite similar in TC and PP soils (Tab. S1). However, PP soil presented a physical constraint to root growth in depth due to the presence of a petrocalcic horizon at 60-65 cm soil deep. The FH soil showed double water availability than TC and PP soils. This could be due to its greater effective depth and lower coarse fragments content respected to the values found in TC and PP soils. Among experimental plots, the soil texture was similar in the three soils and the values of calcium carbonate in the subsoil horizon of TC and PP soils were around 60 %, being 16 % in FH soil (Tab. S1).

In general, soil $N\text{-}NH_4^+$ content was lower than soil $N\text{-}NO_3^-$ content (Tab. 2), probably due to under semiarid conditions, $N\text{-}NH_4^+$ is a low and rather stable fraction of the soil mineral N (Vázquez *et al.* 2006). Soil $N\text{-}NH_4^+$ content was higher in FH plot in deeper depths (15-45 cm) than in TC and PP soils. Soil fertilization combined with climatic conditions in 2014 (Tab. 1) favoured the higher $N\text{-}NH_4^+$

Table 2

Soil $N\text{-}NH_4^+$ and $N\text{-}NO_3^-$ (kg $N \cdot ha^{-1}$) content in 0-15, 15-30, 30-45 and 0-45 cm soil depth from the experimental plots FH (*Fluventic Haploxerepts*), TC (*Typic Calcixerepts*) and PP (*Petrocalcic Palexerolls*) at grapevine bloom during 2013, 2014, 2015 and 2016 seasons

Soil thickness (cm)	2013 FH	2013 TC	2013 PP	2014 FH	2014 TC	2014 PP	2015 FH	2015 TC	2015 PP	2016 FH	2016 TC	2016 PP
					$N\text{-}NH_4^+$ (kg $N \cdot ha^{-1}$)							
0-15	4.52a, A	4.94a, B	3.82a, AB	8.98a, B	10.33a, C	8.93a, C	5.25b, A	3.28a, A	3.23a, A	5.36a, A	4.56a, AB	4.67a, B
15-30	6.31b, B	4.05ab, A	3.74a, A	11.19b, C	9.56b, B	5.83a, B	4.77c, A	3.62b, A	2.84a, A	6.63b, B	4.54a, A	3.33a, A
30-45	4.87b, A	1.96a, A	2.45a, A	8.52b, B	4.96a, B	7.19ab, B	5.52a, A	1.77a, A	5.63a, AB	6.19c, A	2.01a, A	4.07b, AB
0-45	15.70b, A	10.72a, A	9.92a, A	28.70b, B	24.85ab, B	21.94a, B	15.55b, A	8.68a, A	11.71ab, A	18.19b, A	11.11a, A	12.07a, A
					$N\text{-}NO_3^-$ (kg$N \cdot ha^{-1}$)							
0-15	9.76b, A	7.50ab, A	4.54a, A	17.00a, A	10.89a, A	14.10a, B	15.33b, A	5.73a, A	8.90a, AB	20.78b, A	22.06b, B	3.15a, A
15-30	8.67a, A	6.22a, B	3.01a, A	8.11a, A	5.75a, B	11.32a, B	12.09a, A	4.06a, AB	5.97a, AB	5.78b, A	2.46a, A	1.89a, A
30-45	7.64a, A	2.90a, B	2.07a, A	5.47a, A	1.86a, A	3.01a, A	8.99a, A	1.36a, A	2.49a, A	2.87a, A	1.26a, C	1.28a, A
0-45	26.08b, A	16.22ab, A	9.50a, A	30.58a, A	18.50a, A	28.44a, B	36.40b, A	11.15a, A	17.38ab, AB	29.44b, A	25.78ab, B	6.33a, A

For each soil depth and year, different lower case letters indicate significant differences between the three soil types ($p \leq 0.05$). For each soil type, different capital letters indicate significant differences between the four vintages ($p \leq 0.05$). Values are the average of three analyses (n = 3).

concentration in the studied plots throughout the soil profile respect to those values of the other three seasons (Tab. 2). Also, it is observed that PP presents higher levels of $N\text{-}NO_3^-$ (0-30 cm) in 2014 compared with the other years. In 2013 and 2015, total soil $N\text{-}NO_3^-$ availability (0-45 cm) content in the FH plot samples was higher than those of the TC and PP plots, except for PP in 2015. Besides, PP in 2013 and in 2016 showed the lowest soil $N\text{-}NO_3^-$. In 2015, the value in TC was lower than in FH and PP (the latter reaching intermediate values). In the case of the differences between seasons, FH samples did not present significant differences between years for any of the soil depths studied (Tab. 2). Regarding TC soil, season 2016 had the highest soil N-NO_3^- content in the layer 0-15 cm, but at 15-30 cm had lower soil N-NO_3^- values than the other three seasons. In PP samples, soil N-NO_3^- content was higher in 2014, except in 30-45 soil depth, respect to the other seasons, although without significant differences with 2015 season. In summary, it is worth mentioning that the N fertilization done in 2014 allows N-NH_4^+ levels to be strongly increased in all plots and, to a lesser extent, N-NO_3^- content, except for PP, whose values were tripled, indicating that the available mineral N is clearly linked to soil type (Van Leeuwen and De Rességuier 2018). N-NO_3^- levels were similar to those reported by Smith *et al.* (2008), Steenwerth and Belina (2008) and Pérez-Álvarez *et al.* (2015b) in vineyards with Mediterranean conditions.

M u s t o e n o l o g i c a l p a r a m e t e r s a n d y i e l d c o m p o n e n t s : Must and yield parameters from samples of the three different soils studied are shown in Tab. 3. In 2013 and 2015, must from FH soils showed higher pH than TC and PP must. In 2013, 2014 and 2016, FH must presented lower values of probable alcohol and had higher total acidity values due to, probably, its malic acid content. Besides, FH and TC musts showed higher potassium levels than PP must in 2014, 2015 and 2016 seasons, while in 2013, FH samples showed the highest value of this parameter. Colour intensity and TPI values were greater in TC and PP must than those observed in FH must, with the exception of TPI in 2013. Also, total anthocyanins content was higher in TC and PP musts than in FH samples, except in 2013. This result is in agreement with the fact that the vine water status and the N content affects the anthocyanin accumulation and also that a high N level in grapevines inhibited their synthesis, as observed authors as Hilbert *et al.* (2003) and Pérez-Álvarez *et al.* (2017). Meanwhile, Delgado *et al.* (2004) also suggested that N supply decreased the phenolic compound accumulation in 'Tempranillo' grape skin. Therefore, the higher N content (N-NO_3^-) presented in FH soil (Tab. 2) respect to TC and PP soils, along with their greater water availability, could explain the lower total anthocyanins and polyphenol content in FH must respect to TC and PP samples (Tab. 3).

Regarding the yield parameters, in 2014, 2015 and 2016, the weight of 100 berries from FH and TC soils was greater than those from PP soil, being also greater in 2013 in TC plot with respect to the other two soils. The grape yields were greater in TC and PP in 2013, and contrarily, higher in FH in 2014 and 2016. This apparent contradiction generated, especially with the FH grapevines that present the soil with greater effective depth and water availability, was due to the late thinning of bunches, carried out by the vine grower in order to avoid excessive yield that could compromise the grape quality, according to the wine AOC Rioja pattern parameters. The FH soils, which have a greater effective depth, water reserve and nutrients such as N, had higher grape production, except in 2013 and 2015, and provided musts with a higher content of malic acid, potassium and total acidity. In addition, their musts presented color parameters of lower quality compared to TC and PP must. In general, the N application in 2014, produced slight variations in the parameters of the musts, which presented lower values of total acidity and malic acid, lower intensity of color and TPI, with a small increase in the berry size but these effects also can be influenced by the season. Total acidity and pH of the must have also been greatly influenced by the season

Table 3

Must oenological parameters and yield components from three different soils FH (*Fluventic Haploxerepts*), TC (*Typic Calcixerepts*) and PP (*Petrocalcic Palexerolls*) during the four different seasons (2013, 2014, 2015 and 2016)

Soil Type	2013			2014			2015			2016		
	FH	TC	PP	FH	TC	PP	FH	TC	PP	FH	TC	PP
pH	3.28b, A	3.14a, A	3.09a, A	3.28a, A	3.41b, C	3.34ab, C	3.45b, B	3.31a, B	3.26a, B	3.33a, A	3.46b, C	3.28a, B
Probable alcohol (% v/v)	11.81a, AB	12.66b, A	13.10b, A	11.38a, A	14.29b, C	13.66b, B	13.73a, C	13.59a, B	13.68a, B	12.27a, B	13.34b, AB	13.13b, A
Total acidity ($g \cdot L^{-1}$)*	8.48c, B	7.32b, D	6.12a, C	5.69b, A	5.36b, B	4.61a, A	5.80a, A	6.54b, C	5.73a, B	5.84b, A	4.67a, A	4.81a, A
Tartaric acid ($g \cdot L^{-1}$)	6.13b, A	5.11a, A	5.31a, A	6.71b, B	6.66b, C	6.21a, B	7.02b, C	6.77ab, C	6.65a, C	6.84b, BC	6.42a, B	6.57a, C
Malic acid ($g \cdot L^{-1}$)	4.49c, B	3.33b, C	1.99a, B	2.39b, A	2.22b, B	1.21a, A	2.71b, A	3.17c, C	2.18a, B	2.43c, A	1.74b, A	1.20a, A
Potassium ($mg \cdot L^{-1}$)	1824b, B	1377a, A	1226a, A	1565b, A	1663b, B	1212a, A	1771b, B	1756b, B	1475a, B	1416b, A	1403b, A	1150a, A
Colour intensity	6.33a, B	9.67b, B	8.69b, B	3.68a, A	5.92b, A	5.77b, A	4.30a, A	6.08b, A	6.29b, A	3.77a, A	5.41b, A	5.73b, A
TPI**	94.0a, C	99.4ab, C	106.6b, C	70.7a, B	90.7b, BC	89.5b, AB	57.0a, A	73.5b, A	80.8b, A	70.5a, B	86.9b, B	99.0c, BC
Total anthocyanins ($mg \cdot g^{-1}$)	1.61a, C	1.97a, B	1.91a, B	1.32a, B	1.98b, B	1.91b, B	1.00a, A	1.19b, A	1.40c, A	1.33a, B	1.75b, B	1.92b, B
Weight of 100 berries (g)	258.3a, B	280.5b, BC	241.0a, B	302.6b, C	301.2b, C	240.1a, B	231.5b, A	230.5b, A	204.5a, A	243.5b, AB	256.3b ,AB	215.7a, A
Grape yield ($kg \cdot vine^{-1}$)	3.87a, A	5.42b, B	4.93b, C	7.09b, B	3.64a, A	3.91a, A	4.05a, A	3.94a, A	4.04a, AB	7.18b, B	5.11a, B	4.80a, BC

For each parameter and year, different lower case letters indicate significant differences between the three soil types ($p \leq 0.05$). For each soil type, different capital letters indicate significant differences between the four vintages ($p \leq 0.05$). Values are the average of three analyses (n = 3).

(Van Leeuwen *et al.* 2004) and the different parameters of the three soil types also affected the production and the acidity components (Ubalde *et al.* 2007). Therefore, the must composition and vine production of the FH, TC and PP were dependent on their edaphic parameters, such as effective depth, water availability and N status (Choné *et al.* 2001).

Must amino acids composition: The concentrations of the 22 identified and quantified amino acids are shown in Tab. 4. In general, in the four-year period of study, the five amino acids found in highest quantities were Pro, Arg, GABA, Gln, and Glu, while Cit, Gly, Ile, Met, and Lys showed the lowest concentrations. These results are in agreement with those found by other authors (Bell and

Table 4

Amino acids concentration (mg N·kg^{-1}) and yeast assimilable nitrogen (mg N·kg^{-1}) in the musts from the three experimental plots (FH: *Fluventic Haploxerepts*, TC: *Typic Calcixerepts*, and PP: *Petrocalcic Palexerolls*) during the four studied vintages (2013, 2014, 2015 and 2016)

	2013			2014			2015			2016		
	FH	TC	PP	FH	TC	PP	FH	TC	PP	FH	TC	PP
Pro	35.05b, B	28.19b, B	16.07a, AB	16.52a, A	35.32b, C	24.79a, C	30.27a, B	17.92a, A	19.89a, BC	11.36a, A	14.59b, A	11.19a, A
Arg	80.09c, D	41.22b, D	4.33a, A	36.17b, C	21.08a, C	6.48a, A	24.18b, B	6.57a, A	7.20a, A	11.05b, A	10.41b, B	4.50a, A
GABA	13.50c, C	8.16b, C	3.71a, B	7.62b, B	10.29c, D	5.17a, C	5.78c, AB	3.46b, A	2.76a, A	4.86a, A	6.09b, B	4.75a, C
Gln	16.69b, C	15.52b, B	5.18a, A	9.51a, B	9.02a, A	8.92a, B	9.08b, AB	7.07a, A	7.20a, AB	7.04ab, A	8.92b, A	5.94a, A
Glu	5.04b, B	4.46b, B	2.75a, A	2.75a, A	3.13a, A	3.05a, A	4.80b, B	3.07a, A	2.78a, A	4.58a, B	5.99b, C	4.77a, B
Ala	5.81c, C	4.12b, C	1.73a, A	4.40b, B	3.04a, B	2.40a, B	3.15b, A	1.61a, A	1.65a, A	3.52b, A	3.72b, C	2.82a, B
His	9.41c, B	4.67b, D	0.59a, A	2.32b, A	2.52b, B	1.32a, B	2.11b, A	0.84a, A	0.65a, A	1.93a, A	3.62b, C	2.35a, C
Trp	3.88b, B	4.31b, C	1.80a, C	2.12b, A	2.90c, B	1.42a, B	1.89c, A	1.49b, A	1.04a, A	2.13a, A	3.23b, B	1.94a, C
Thr	2.97c, B	2.63b, C	1.16a, A	1.98b, A	1.89b, B	1.26a, A	1.84b, A	1.23a, A	1.08a, A	1.85b, A	2.06b, B	1.35a, A
Ser	3.14b, B	2.75b, C	1.39a, A	1.99a, A	2.31a, B	1.88a, BC	2.27b, A	1.60a, A	1.63a, AB	2.05a, A	2.46b, B	2.00a, C
Asp	1.71b, B	2.01b, C	1.33a, C	1.32b, A	0.96ab, A	0.69a, A	1.71a, B	1.59a, B	1.48a, C	1.54b, AB	1.56a, A	0.97a, B
Cys	1.71c, C	0.77b, D	0.15a, A	0.31b, A	0.34b, B	0.09a, A	0.25b, A	0.07a, A	0.17ab, A	0.51a, B	0.61a, C	0.55a, B
Phe	0.83c, C	0.57b, B	0.25a, A	0.38a, AB	0.48b, B	0.30a, A	0.34b, A	0.19a, A	0.24a, A	0.44a, B	0.55b, B	0.43a, B
Val	1.04b, B	0.87b, B	0.37a, A	0.57a, A	0.84b, B	0.59a, B	0.60b, A	0.35a, A	0.40a, A	0.73a, A	1.13b, B	0.87ab, C
Leu	0.81b, B	0.65b, B	0.28a, B	0.37a, A	0.57b, B	0.34a, B	0.29b, A	0.17a, A	0.17a, A	0.42a, A	0.75b, B	0.56ab, C
Tyr	0.44c, C	0.23b, B	0.03a, A	0.21b, AB	0.23b, B	0.09a, B	0.17b, A	0.05a, A	0.06a, A	0.25b, B	0.32c, C	0.17a, C
Asn	1.10c, C	0.80b, B	0.17a, B	0.27b, A	0.23b, A	0.08a, A	0.73c, B	0.37b, A	0.20a, B	0.43a, A	0.69b, B	0.40a, C
Cit	1.12b, C	1.01b, B	0.17a, B	0.42b, A	0.41b, A	0.00a, A	0.41b, A	0.14a, A	0.14a, B	0.72b, B	0.83c, B	0.30a, C
Gly	0.77c, C	0.37b, C	0.02a, A	0.21a, A	0.32b, BC	0.13a, A	0.39a, B	0.27a, B	0.44a, B	0.15a, A	0.19b, A	0.16ab, A
Ile	0.37b, B	0.34b, AB	0.17a, A	0.25a, A	0.41b, AB	0.29ab, A	0.23b, A	0.13a, A	0.15a, A	0.31a, AB	0.60a, B	0.33a, A
Met	0.26c, B	0.07b, AB	0.00a, A	0.12b, A	0.10ab, B	0.06a, B	0.16b, A	0.05a, A	0.03a, B	0.24b, B	0.30c, C	0.19a, C
Lys	0.32c, C	0.19b, C	0.07a, A	0.17ab, B	0.10a, B	0.22b, C	0.08c, A	0.02a, A	0.04b, A	0.16b, B	0.17c, C	0.13a, B
Taa	186.05c, C	123.72b, D	41.73a, A	89.95a, B	96.50b, C	59.59a, B	90.73b, B	48.26a, A	49.40a, AB	56.23a, A	68.41b, B	46.66a, AB
Taa without Pro	151.00c, C	95.53b, C	26.66a, A	73.44b, B	61.17b, B	34.80a, AB	60.46b, AB	30.35a, A	29.51a, A	44.88a, A	53.82b, B	35.47a, B
YAN	117.88c, B	69.42b, B	32.09a, A	86.81b, A	80.10ab, B	66.79a, C	86.54 b, A	42.41 a, A	50.25 a, B	79.25 b, A	110.49 c, C	55.40 a, B

For each year, different lower case letters indicate significant differences between the three soil types ($p \leq 0.05$). For each soil type, different capital letters indicate significant differences between the four vintages ($p \leq 0.05$). Taa = Total amino acids. YAN = Yeast assimilable nitrogen. Values are the average of three analyses (n = 3).

Henschke 2005, Garde-Cerdán *et al.* 2014, Pérez-Álvarez *et al.* 2017).

Regarding the differences found each year depending on the soil type, in 2013, the must from FH soil presented the highest concentration of 12 amino acids (Arg, GABA, Ala, His, Thr, Cys, Phe, Tyr, Asn, Gly, Met, and Lys), while the samples from PP soil showed the lowest content of all amino acids (Tab. 4). The levels of amino acids in TC soil were intermediate between FH and PP samples, although there were not significant differences with FH soil for Pro, Gln, Glu, Trp, Ser, Asp, Val, Leu, Cit, and Ile. Therefore, total amino acids, total amino acids without Pro and YAN in FH samples had the highest values, TC samples had intermediate levels, and PP musts had the lowest content of these three parameters (Tab. 4). In this sense, FH soil, which, as previously mentioned, has the greatest effective depth and water reserve available (Tab. S1), showed higher $N\text{-}NH_4^+$ and $N\text{-}NO_3^-$ soil availability at 0-45 cm depth than the other two soils (Tab. 2) and so the FH musts have more amino acid contents than the two other soil types, TC and PP, in rainier years than the average. It is noteworthy that YAN highest values were obtained for FH samples. However, musts from the different soils and years were below the threshold for appropriate fermentation (Bely *et al.* 1990, Bell and Henschke 2005). Moreover, Van Leeuwen *et al.* (2000) suggested that the YAN values of the must are an indicator of grapevine N status, and besides they have used them to make N fertilization decisions in some vineyards in California (Lee and Schreiner 2010).

In 2014, TC must had, in general, higher amino acids concentration than the other two soils, highlighting the content of Pro, GABA, Trp, Phe, Val, Leu, and Gly (Tab. 4). Meanwhile, FH must showed the highest Arg and Ala content. PP must presented the lowest concentration of the most of the amino acids. Total amino acids content was the highest in TC samples, with no significant differences between FH and PP samples (Tab. 4). Regarding total amino acids without Pro, PP samples had the lowest content. The musts from FH soil presented higher YAN content than PP samples and TC musts had intermediate YAN values closer to FH. This fact could be related with the higher soil $N\text{-}NH_4^+$ content observed in FH vineyard respect to the PP soil (Tab. 2). This $N\text{-}NH_4^+$ mineralized can be easily transformed, throughout the grapevine cycle, to $N\text{-}NO_3^-$ content, which is the form of N available for the grapevine. On the other hand, in 2014, there was no clear relation between fertilization and increase in the content of N compounds in musts, except PP, probably caused by its lower effective depth and the initial N status of the vines (Bell and Henschke 2005).

In 2015, the highest content of all amino acids was found in the FH samples, with the exception of Pro, Asp, and Gly, which did not show significant differences between soils, and Cys, which content only showed differences with TC samples (Tab. 4). Consequently, total amino acids, total amino acids without Pro and YAN were higher in FH soil than in the other two soils. Probably, the highest N compounds content in FH must with respect to the other two samples, could be related to the cluster thinning operation and to the weather conditions of this season (Tab. 1). It is possible that these conditions favored a better rate of organic N mineralization in this soil; thus FH soil had higher $N\text{-}NO_3^-$ content in the soil surface layer than the other soils (Tab. 2), being the content of $N\text{-}NO_3^-$ in soil surface at bloom considered representative of the N availability for the grapevine, which it is correlated with grapevine vigor development and yield (Pérez-Álvarez *et al.* 2013).

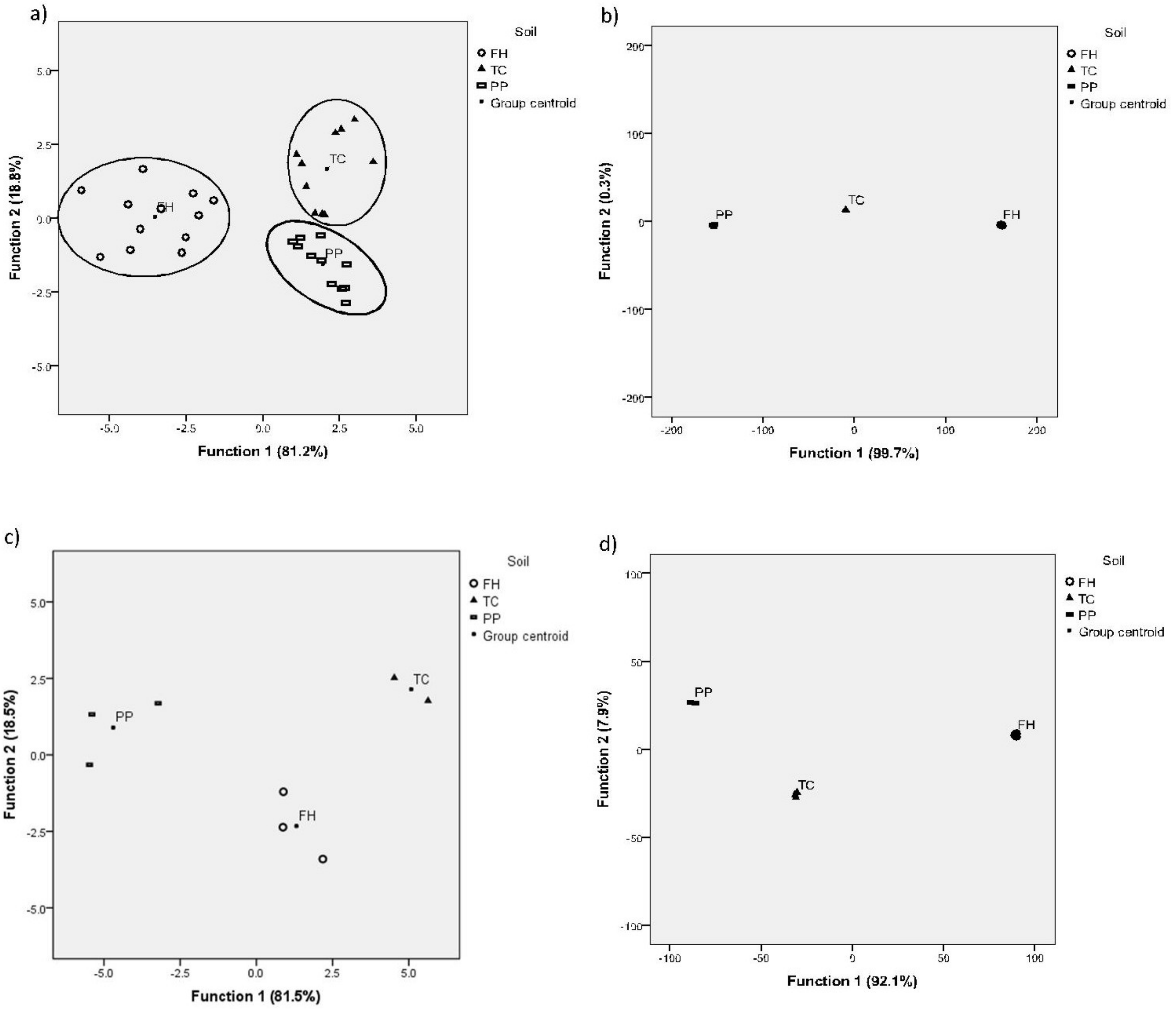
The influence of the fertilization in 2014 was observed in the must from TC soil in 2016, with the highest content of must amino acids, with the exception of Arg, Gln, Ala, Thr, Asp, Cys, Leu, Gly, and Ile (Tab. 4). Regarding the total amino acids, total amino acids without Pro contents and YAN, also the highest values were found in the musts from TC soil. This fact could probably be related to the higher levels of nitrates found in the TC vineyard at the surface level (0-15 cm) (Tab. 2).

With respect to the differences found for each soil type as a function of vintage, the musts from FH soil had the highest concentration of most amino acids in 2013 (Tab. 4). This must in 2013 had the highest total amino acids and total amino acids without Pro contents, which were more than two-fold (in the case of 2014 and 2015) and three-fold (2016) than in the other seasons. This could be related to the behavior of this type of soil depending on the rainfall pattern of the season, it would be a response dependent on the interaction between the edaphic parameters of FH and climatic conditions. Therefore, 2013 was a rainy season (Tab. 1), and must from FH showed the highest content of amino acids; 2014 and 2015 were intermediate years both in rainfall and in must amino acids concentration; and in 2016, there was a considerable decrease in rainfall throughout the grapevine vegetative cycle and also the amino acids decreased in this FH must. Moreover, identical behavior has been observed with respect to the YAN.

In the samples from TC soil, the highest concentration of several amino acids was found in 2013, *i.e.* Arg, Gln, His, Trp, Thr, Ser, Asp, Cys, and Gly; Pro, and GABA in 2014, and Glu, Tyr, and Met in 2016 (Tab. 4). The lowest content of most amino acids was found in 2015, *i.e.* Arg, GABA, Ala, His, Trp, Thr, Ser, Cys, Phe, Val, Leu, Tyr, and Cys, and Gly in 2016. Total amino acids and total amino acids without Pro were higher in 2013 and throughout the study, these parameters content decreased in must samples. On the contrary, in the fourth season, total N concentration increased, being also the YAN concentration the highest found in this soil. Therefore, the effect of N fertilizer applied in 2014 on this vineyard was appreciated in the must N compounds in 2016.

In the musts from PP soil, the highest content of several amino acids was found in 2016, *i.e.* Glu, His, Cys, Phe, Val, Leu, Tyr, Asn, Cit, and Met; Gly in 2015, and Lys in 2014 (Tab. 4). The lowest concentration of Met was found in 2013, of Asp, Asn, and Cit was found in 2014, and of GABA, Trp, and Leu in 2015. In the case of this soil, total amino acids content and YAN was the lowest in 2013, and total amino acids without Pro was only significantly higher in 2016, with no differences to 2014 values. Thus, this PP soil presented a different behavior with respect to the other two soils, probably due to its lower effective depth, and lower available water retention capacity, since it contributed very little to YAN contents in the rainy year (2013), and slightly more in drier years (2014 and 2015). The low contents in YAN in TC musts can also be attributed to the lower N availability for the vine due to the high percentages of calcium carbonate in this type of soil (Reynolds 2010). In addition, in 2013, 2014, 2015 and 2016 seasons, due to their low content in YAN, less than 100 mg N·kg^{-1}, the PP musts presented problems in the kinetics of alcoholic fermentation, being winemaking much slower than in the other samples (data not shown). However, similar as occurred with TC soils, an increase of YAN was observed in 2016 as a response to the N fertilization carried out in 2014.

D i s c r i m i n a n t a n a l y s i s : In order to classify the different samples, discriminant analysis was performed on data expressing the concentration of amino acids in the different must samples, taking into consideration the soil or the vintage as classification factors (Figs 1 and 2, respectively). For soil type as factor, bearing in mind all the studied years (Fig. 1a), function 1 explained 81.2 %



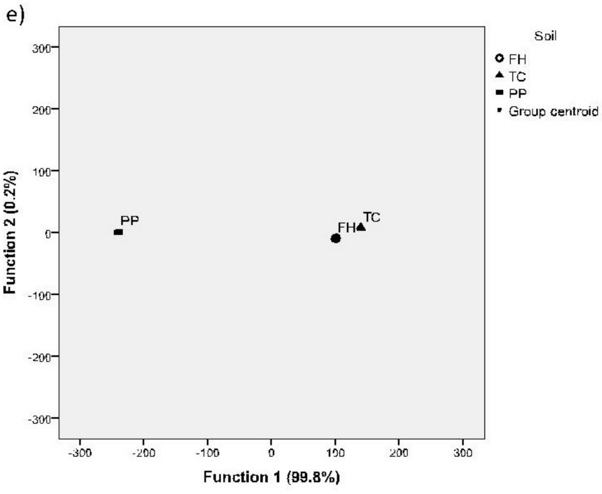


Fig. 1: Application of discriminant analysis to the must amino acid concentrations, with soil type as classification factor (FH: *Fluventic Haploxerepts*, TC: T*ypic Calcixerepts* and PP: *Petrocalcic Palexerolls*): **a**) the whole seasons, **b**) 2013, **c**) 2014, **d**) 2015, and **e**) 2016.

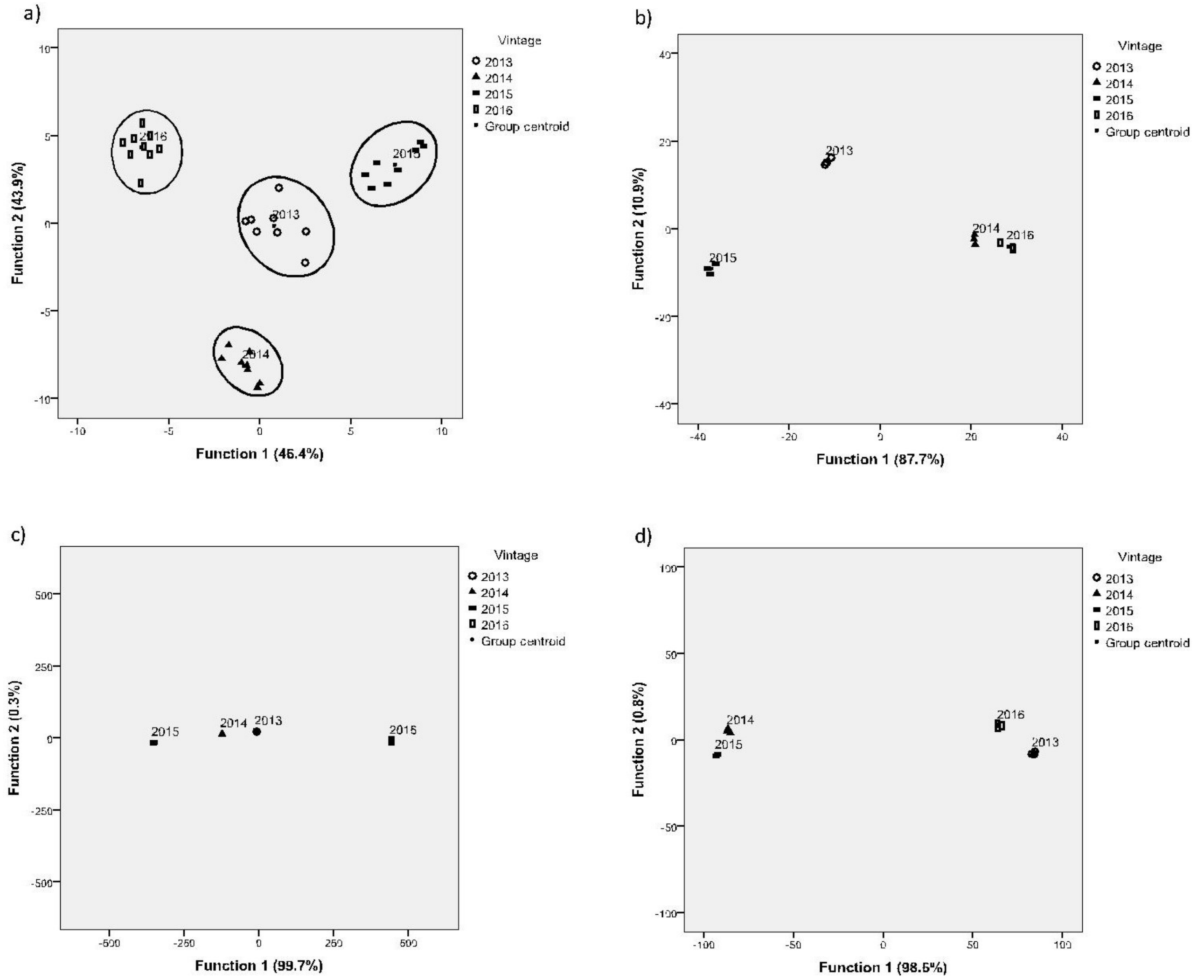


Fig. 2: Application of discriminant analysis to the must amino acid concentrations, with vintage as classification factor (2013, 2014, 2015 and 2016): **a**) the whole soil types, **b**) FH: F*luventic Haploxerepts*, **c**) TC: *Typic Calcixerepts*, and **d**) PP: *Petrocalcic Palexerolls.*

and function 2 explained 18.8 %, so the total of variance explained was 100 %. The variables that contributed the most to the discriminant model were Thr, Trp, and Gln (function 1) and Arg, Thr, and Lys (function 2). The discriminant model showed a good separation among different soil types. Regarding to 2013 samples (Fig. 1b), function 1 explained a very high percentage of variance (99.7 %) and function 2 explained only 0.3 % (total variance = 100 %). The variables that contributed the most to the discriminant model were Cit, Trp, and Gln (functions 1 and 2). The discriminant model showed a very good separation among different soils. Fig. 1c shows that there was a good separation among different soils for 2014 vintage, the function 1 explained 81.5 % of the variance and function 2 explained 18.5 % (cumulative total of 100 %). Cys, and Pro were the main contributors to the discriminant model (functions 1 and 2). As for 2015 (Fig. 1d), function 1 explained 92.1 % of the variance and function 2 represented 7.9 % (100 % cumulative total). The discriminant function showed a very good separation among the three different soil types. The variables that contributed the most to the discriminant model were GABA, Gln, and Asn (function 1) and Gln, Asn, and GABA (function 2). Fig. 1e shows the results for 2016. Function 1 explained 99.8 % of the variance and function 2, only 0.2 % of the variance (100 % of the total). The discriminant function showed a good separation among the PP and the other two soils (FH and TC). Cit, Tyr, and Lys were the variables that contributed the most to the discriminant model (function 1).

For vintage as factor, the discriminant function showed a very good separation among the years for all samples (Fig. 2a). Function 1 explained 46.4 % of the variance and function 2 represented 43.9 % (90.4 % cumulative total). The variables that contributed the most to the discriminant model were His, Cys, and Gly (function 1) and Thr, His, and Glu (function 2). Regarding to FH soil (Fig. 2b), function 1 explained a very high percentage of variance (87.7 %) and function 2 explained only 10.9 % (total variance = 98.6 %). Ala, Arg, and Glu were the variables that contributed the most to the discriminant model (function 1) and Ala, Cys, and Glu those of the function 2. Fig. 2c shows the results for TC soil, function 1 explained 99.7 % and function 2 explained 0.3 %, so the total of variance explained was 99.9 %. The variables that contributed the most to the discriminant model were Tyr, Pro, and Trp (function 1) and Phe, Trp, and Pro (function 2). For PP soil (Fig. 2d), function 1 explained a very high percentage of variance (98.6 %) and function 2 explained only 0.8 % (total variance = 99.4 %). Pro, Trp, and Asn were the variables that contributed the most to the discriminant model (function 1).

The percentages of must amino acids and YAN variance attributable to soil, season and their interactions are presented in Tab. S2. All the N compounds studied show a very high dependence of both, soil and season, as well as their interaction, except in the case of Pro content that is little influenced by soil, Ile that it is little influenced by season, less by soil and nothing by their interaction. In the case of Asp content in musts, the interaction of the two factors studied show low response. Stand out the high influence of the soil type on Ala, Thr, and Tyr contents in musts. These amino acids are important for grape and wine quality. Ala is one of the majority amino acids in the grape (Bell and Henschke 2005) and moreover it is a good nitrogen source for yeast (Gobert *et al.* 2019), therefore it contributes to the correct development of alcoholic fermentation. Thr and Tyr are precursors of fermentative volatile compounds, n-propanol and tyrosol, respectively, and therefore, they contribute to the wine aromatic quality (Garde-Cerdán and Ancín-Azpilicueta 2008).

Conclusions

The identification and quantification of grape amino acids and YAN allowed us to differentiate must samples from three different soil types, in relation to their edaphic properties, especially soil fertility, calcium carbonate accumulation, effective depth and available water reserve. Moreover, grape amino acids and YAN concentration in must allowed us to differentiate, in each soil type, the samples of each season. In general, content of total amino acids with and without Pro in musts depended on the soil type, season and their interaction.

The fertile soils, such as FH, with high effective depth and water reserves, produced musts with a higher amino acids content and YAN, in rainy years. Although, in years with precipitations considered normal or below the average, these soils produced musts with a low content of amino acids. PP soil, with less effective depth and fertility, did not have this behavior, as it did not show a significantly positive response to a greater availability of rainwater in the soil profile. Finally, TC soils produced musts with lower amino acid content in years considered climatically normal.

Consequently, it is important to know the soil properties as well as its N available content, in order to apply corrective actions in the vineyard, if it is necessary, avoiding subsequent problems in the cellar due to the lack of N.

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